

AD _____

Award Number: W81XWH-FE-0001 F

TITLE: VÖÖÖ^æÖ^}^Á[[^ { [!] @ { • Á Á [[á Á [^! * á Á ^! • • Á [] È [[á Á [^! * á Á
Ò [• á [] @ á Á • [] @ á á

PRINCIPAL INVESTIGATOR: Ö: ÄÖæãÁ [ä^

CONTRACTING ORGANIZATION: University of Öä [!] äÄÜä Ää* [Üä Ää* [ÈÖÖÁ GEJHÁ

REPORT DATE: U& ä^! Á GFG

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY) 01-10-2012		2. REPORT TYPE Annual		3. DATES COVERED (From - To) 15 Sep 2011 - 14 Sep 2012	
4. TITLE AND SUBTITLE TGF-Beta Gene Polymorphisms in Food Allergic versus Non-Food Allergic Eosinophilic Esophagitis				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-11-1-0741	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) David Broide MB ChB Seema Aceves MD PhD				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of California San Diego, La Jolla, CA 92093-0635				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S.Army Medical Research and Material Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The diagnosis of eosinophilic esophagitis (EoE) is based on the presence of ≥ 15 eosinophils/hpf in the esophagus of a patient with symptoms of esophageal dysfunction in whom GERD is excluded. EoE is likely mediated by interaction of environmental allergens (such as foods) with several genes. Food antigens play an essential role in EoE since specific food elimination diets and amino acid formulas successful EoE therapy in 60-98% of subjects. Indeed, the majority of children with EoE have specific IgE to foods but they often continue to ingest these foods due to lack of immediate hypersensitivity reactions. This study focuses on the gene-environment interaction of food consumption in food sensitized children with EoE and the TGFb1 genes. We hypothesize that in EoE there is a gene polymorphism (TGFb1) environment (food) interaction that contributes to increased IgE mediated TGFb1 expression in the esophagus and increased esophageal remodeling in a subset of EoE subjects. As esophageal stricture formation is an important complication of remodeling in EoE (6-12% of children; 33% of adults), identifying genetic polymorphisms in TGFb1 in EoE may allow the early identification of food sensitized children at risk for the development of this significant complication of EoE.					
15. SUBJECT TERMS Eosinophils, TGF-b, remodeling, food allergy					
16. SECURITY CLASSIFICATION OF: U			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 6	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	6
Reportable Outcomes.....	6
Conclusion.....	6
References.....	6
Appendices.....	6

Introduction:

The diagnosis of eosinophilic esophagitis (EoE) is based on the presence of $> \sim 15$ eosinophils/hpf in the esophagus of a patient with symptoms of esophageal dysfunction (i.e. dysphagia, anorexia, early satiety, failure to thrive) in whom gastro-esophageal reflux disease has been ruled out by lack of response to treatment with a proton pump inhibitor. The prevalence of EoE is approximately 1:2,500 in pediatric populations the focus of this study. Although there is evidence of a familial association of EoE, EoE as with other food allergic and allergic diseases is likely mediated by interaction of environmental allergens (such as foods) with several genes. Food antigens play an essential role in EoE since specific food elimination diets and amino acid formulas successful EoE therapy in 60-98% of subjects. Indeed, the majority of children with EoE have specific IgE to foods but they often continue to ingest these foods due to lack of immediate hypersensitivity reactions. This study focuses on the gene-environment interaction of food consumption in food sensitized children with EoE and the TGF β 1 genes. We hypothesize that in EoE there is a gene polymorphism (TGF β 1) environment (food) interaction that contributes to increased IgE mediated TGF β 1 expression in the esophagus and increased esophageal remodeling in a subset of EoE subjects. As esophageal stricture formation is an important complication of remodeling in EoE (6-12% of children; 33% of adults), identifying genetic polymorphisms in TGF β 1 in EoE may allow the early identification of food sensitized children at risk for the development of this significant complication of EoE. The TGF- β 1 gene promoter has a well characterized functional SNP C-509T which results in three genotypes, i.e. TT, CT, or CC. Prior studies have demonstrated that the TT genotype of the TGF- β 1 gene is associated with increased levels of fibrosis in progressive kidney disease. We will examine whether the TT genotype is associated with increased fibrosis in food sensitized EE subjects. We hypothesize that the TGF- β TT genotype is associated with increased levels of TGF- β expression in the esophagus in food sensitized EE and promotes the development of esophageal remodeling.

Body:

This proposal outlines 6 tasks to be completed during the three year proposal. In year 1 of this proposal we have completed task 1 and worked on tasks 2-4 (to be completed by month 33) as planned in our original proposal.

Task 1: Approval for human subjects studies (month 1-6)

Revisions to the human subject's consent forms were made as requested by DOD. UCSD IRB approval of these modified consents was obtained on April-5-2011.

We received an approval letter from DOD permitting us to start working on the project on April 13, 2011 in an e-mail from Ms Duchesneau (Chief, Human Subjects Protection Review; Caryn.Duchesneau@us.army.mil).

The protocol and consent was reviewed by the UCSD IRB on 9-22-11 and approved through 9-21-12.

Continuing review documents will be submitted to the UCSD IRB in August 2012 to continue approval for this project beyond Sept 21, 2012.

Task 2: Enrolling EE (Food IgE+ and Food IgE-) subjects (n= 400 subjects) (month 1-33)**2a) Database for EE genotyping clinical trial established (month 1)**

The database for EE genotyping has been established.

There have been 55 new subjects enrolled since September 2011, which will meet the annual goal of 60 new patients annually. Combining this with our previously accumulated patient population brings the total number of EoE subjects in the database to 387. This database has been generated and subject genotypes are entered into this database on an ongoing basis (see below).

2b) Demographic and clinical information entered (month 1-33)

Demographic and clinical information for all new subjects (n= 55) is entered into the database on a weekly basis. Similar data is available from previously entered subjects

2c) Results of upper GI endoscopy entered (month 1-33)

Upper GI endoscopy results is entered on a weekly basis for all new subjects (n= 55)

2d) Results of esophageal biopsy eosinophils/hpf entered (month 1-33)

Pathology reports are generated in 2 days following the endoscopy with biopsy and results are entered into the database on a weekly ongoing basis.

Task 3: Quantitating esophageal expression of TGF-b and pSmad in EE (Food IgE+ and Food IgE-) (month 1-33)

3a) Making pathology blocks of esophageal biopsy (month 1-33)

Esophageal biopsy blocks are made following each endoscopy and biopsy (n= 55)

3b) Sectioning esophageal biopsy blocks (month 1-33)

Sectioning of esophageal biopsy blocks is ongoing on a bi-weekly basis (n=55). Sections are first evaluated for the presence of lamina propria (LP) and those with LP are preferentially evaluated.

3c) TGF-b immunostain: To quantitate TGF-b+ cells (month 1-33)

Quantitation of TGFb+ cells. Immunohistochemistry and quantitation is ongoing. To date 57 EoE biopsies from the 387 EoE subject database with adequate lamina have been evaluated for TGFb+ cells. Preliminary data shows increased numbers of TGFb1 expressing cells in genotype TT subjects ($p<0.001$). More subjects must be enrolled (to be accomplished in months 13-33) before analysis of phenotype and genotype can be appropriately completed.

3b) pSmad immunostain : To quantitate pSmad+ cells (month 1-33)

Immunohistochemistry and quantitation is ongoing. To date 20 EoE biopsies with adequate lamina propria from the 387 EoE subject database have been evaluated for pSmad+ cells.

3e) MBP Ab; Immunostain to quantitate eosinophils (month 1-33)

Evaluation of MBP is not yet started and eosinophils have been quantitated using hematoxylin and eosin staining. Quantitation of eosinophils shows correlation between the numbers of TGFb1 positive cells and eosinophils. More subjects are required prior to making definitive conclusions

3f) Control Abs (month 1-33)

Control Abs are routinely used to immunostain slides to exclude non-specific staining of tissues. We have not noted any non-specific staining with either the control Abs for TGF-b1 Ab or pSmad.

3g) Slides (month 1-33)

Esophageal tissue sections are sectioned onto slides on a routine basis

3h) Results of esophageal biopsy histology (task 3a-g) entered into the database.

Esophageal histology results are entered into the database on a weekly basis.

Task 4: TGF-b genotyping in EE (month 1-33)

4a) Consent for genotyping in EE subjects (month 1-33)

EoE subjects are consent weekly on an ongoing basis. To date 52 new EoE subjects have been consented for genotyping meeting the annual goal of 50 per year. Our collaborators remain willing to provide additional subject specimens for genotyping if needed.

4b) TGF-b genotyping (month 1-33)

Genotyping is ongoing and occurs in batches. 52 additional subjects have been consented for genotyping since September 2011, 32 have been genotyped, and 20 are pending genotyping meeting the annual goal of 50 per year (by September 2012).

4c) TGF-b single nucleotide polymorphisms (SNP) information entered into database (month 1-33)

SNP information is entered into the database on an ongoing basis

4d) Results of TGF-b SNP genotyping, phenotyping, and TGF-b/pSmad analysed

Analysis is ongoing and larger numbers are needed to make definitive conclusions regarding genotype and phenotype. As noted above, TT genotype subjects have quantitatively more TGFb1 positive cells than CC or CT subjects ($p<0.001$)

Task 5: Quantitating esophageal remodeling in EE (Food IgE+ and Food IgE-) (month 28-33)

5a) Trichrome stain: To quantitate fibrosis

Trichrome staining has not yet begun as it is scheduled from months 28-33 (month 28-33)

5b) H and E stain: To quantitate basal zone hyperplasia (month 28-33)

Basal zone hyperplasia quantitation has begun and completed on all subjects whose TGFb1 counts have been assessed (n=57) and continues. Note that this task was not planned to begin until month 28.

5c) VWF Immunostain: To quantitate blood vessels (month 28-33)

Not yet started, to begin in month 28.

5d) VCAM Immunostain: To quantitate activation of blood vessels (month 28-33)

Not yet started, to begin in month 28.

5e) Control abs (month 28-33)

Control Abs are used to immunostain slides to exclude non-specific staining of tissues.

5f) Slides (month 28-33)

Esophageal tissue sections are sectioned onto slides on a routine basis

Task 6: Preparation of manuscript (month 33-36)

Manuscript preparation has not begun

Key Research Accomplishments:

- Enrollment of 55 new EoE subjects (results in 387 total EoE subjects in the database)
- Enrollment of 52 EoE subjects for genetic studies
- Genotyping of 32 EoE subjects since September 2011
- Analysis of TGFb1 and pSMAD immunostaining in 57 and 20 subjects, respectively

Reportable Outcomes:

There are currently no reportable outcomes in the first 12 months of working on this project.

Conclusion:

There are currently no conclusions.

References:

None.

Appendices:

None.

Manuscripts/Reprints, Abstracts:

None.